UNIVERSITY OF VIRGINIA
HEALTH SCIENCES CENTER
LYMPHOCYTE CULTURE CENTER

June 23, 1993

Dr. Peter Heymann
MR 4, Box 29
Health Sciences Center

Dear Peter:

The Lymphocyte Culture Center (LCC) is a research support facility of the University of Virginia School of Medicine. The primary function of the LCC is to make available to researchers the most current technology and expertise for the construction of lymphocyte-myeloma hybrids for the production of monoclonal antibodies. The LCC can also provide expertise in the use of these reagents in basic research programs.

The center occupies an approximately 510 square foot modern laboratory equipped with cell culture incubators, laminar flow hoods, centrifuges, inverted and standard microscopes, automated microplate washer and ELISA plate reader, etc. We also have facilities for the cryopreservation of established hybrids and myeloma cell lines in either liquid nitrogen or at -80°C. The LCC is currently staffed by myself, a laboratory specialist C, a laboratory specialist A and a part-time laboratory aide.

During the past ten years of operation, the LCC has successfully completed a number of projects for investigators throughout the University and the Medical School. We have also successfully completed a contract with the Federal Bureau of Investigation involving the production and characterization of monoclonal antibodies for forensic applications. The hybridomas secreting these antibodies have been patented and kits employing these reagents for forensic diagnosis are commercially available. We routinely obtain fusion frequencies greater than 85% (wells containing viable hybrids/wells plated x 100) or approximately one in every 2×10^5 spleenocytes forming a viable hybrid. As a result of these high fusion frequencies, we have been able to construct hybridomas secreting monoclonal antibodies specific to the immunogen from a single fusion in most of the projects that we have initiated.

Our standard mouse fusion protocol (M.D. Chapman, W.M. Sutherland, and T.A.E. Platts-Mills (1984), J. Immunol. 133:2488-2495) is modified from published procedures (Oi, V.T. and Herzenberg, L.A. in Selected Methods in Cellular Immunology, ed. B.B. Mishell and S.M. Shiigi, W.H. Freeman & Co., 1980, p. 351). Briefly, spleen cells from an immunized mouse are washed once in Iscove's MDM and mixed with washed Sp2/0-Ag14 myeloma cells (Shulman, et al. (1978), Nature 276:269) at a 5:1 ratio (spleenocyte to myeloma). The cells are pelleted and the medium aspirated. Cell fusion is accomplished by the stepwise addition of 37% polyethylene glycol (PEG 1,000, Koch-Light) over one minute. The PEG is then diluted dropwise with 10 ml Iscove's MDM and the cells pelleted and gently washed once in Iscove's MDM containing 15% selected fetal bovine serum, hypoxanthine (H), and thymidine (T). The cells are resuspended in this HT medium, transferred to a petri dish and incubated at 37°C in a humidified

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atmosphere of 5% CO_2/95% air for one hour. The cells are then resuspended in HT medium and plated into 96-well tissue culture plates (Costar, Cambridge, Mass.) at a density of approximately 2-4 x 10^5 cells/well. The cultures are fed 24 hours later with HT medium containing aminopterin (HAT medium) and maintained in this medium for two weeks. Macroscopic colonies usually appear within 7-10 days following the fusion and supernatants are screened for specific antibody production. Positive hybrids are cloned twice by limiting dilution, and frozen cell stocks are stored in liquid nitrogen cell banks. We also routinely generate bulk monoclonal antibody as either ascites, or as culture supernatants in either fetal bovine serum containing medium or in a defined medium (protein free). The LCC also provides the routine purification of monoclonal antibodies by affinity chromatography on engineered recombinant Protein G columns.

In addition to providing all of the cell culture aspects of hybridoma production, we can also assist in developing assay procedures tailored to your specific needs. Enzyme linked immunosorbent assays (ELISA) [Engvall, E. and Perlman, P., "Enzyme linked immunosorbent assay (ELISA): quantitative assay for immunoglobin." Immunochem. 8:871-874 (1971)] are routinely performed in this laboratory for antigen specific antibody production, antibody class and subclass identification, and non-specific immunoglobulin production and quantitation [Engvall, E. and Pesce, A.J., eds. Quantitative Enzyme Immunoassay, Scad. J. Immunol. (Suppl. &) 1978]. These assays may be either qualitative or quantitative (using our Titertek Multiskan MC microplate reader, Flow Laboratories) and form an integral part of the services provided by the LCC.

Please contact me if I can be of further assistance to you in meeting the goals of your research projects.

Sincerely,

Bill

William M. Sutherland, Ph.D.
Associate Director
Lymphocyte Culture Center
Research Associate Professor of
Anatomy and Cell Biology

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